DEVELOPMENT OF A SORGHUM CORE COLLECTION: REFINEMENT AND EVALUATION OF A SUBSET FROM SUDAN[1]

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Dahlberg, J. A. *(National Grain Sorghum Producers, 4201 North I-27, Lubbock, TX 79403; e-mail jeff@sorghumgrowers.com),* **J. J. Burke** *(USDA-ARS Plant Stress and Germplasm Development Unit, 3810 4th Street, Lubbock, TX 79415),* **and D. T. Rosenow** *(Texas Agricultural Experiment Station, Route 3, Box 219, Lubbock, TX 79401-9757).* DEVELOPMENT OF A SORGHUM CORE COLLECTION: REFINEMENT AND EVALUATION OF A SUBSET FROM SUDAN. *Economic Botany 58(4):556–567, 2004.* *The U.S. National Plant Germplasm System (NPGS) maintains over 40 000 sorghum accessions, which is too large to adequately screen at one time. The objectives of this research were to establish an initial core collection and use clustering techniques to refine one sub-set of that core. Because much of the total sorghum collection lacks complete descriptor or other usable data, the collection was broken out into its respective country of origin and random selection of 10% of the accessions from each country was used to develop the core. Based on these criteria, a core collection of 3011 accessions representing 77 different countries was developed. A more complete phenotypic dataset representing the Sudan allowed for the development of a refined subset of the core collection from this country. The core from Sudan is made up of 352 accessions that represent 13.8% of the total Sudan collection. This core was then evaluated for imbibitional high temperature sensitivity, leaf high temperature sensitivity, and acquired thermotolerance. The results show that these assay systems evaluate distinct aspects of the sorghums' metabolism and that each assay provides unique information about the relative heat tolerance of the sorghum. The impact of the sorghum core collection at this time is unknown. Theoretically, the core should provide a road map from which scientists can map genetic diversity of sorghum and further enhance the capabilities to isolate and clone genes of importance in the future.*

Key Words: Sorghum, *Sorghum bicolor,* germplasm, Sudan, heat tolerance, genetic diversity.

Sorghum [*Sorghum bicolor* (L.) Moench] was planted on 3.8 million ha in the United States in 2003 with an average yield of 3305 kg ha^{-1} (USDA 2004). Sorghum is valued at approximately 2.0 billion dollars annually. Prior to hybrid development in the USA, sorghum improvement relied heavily on mutants from primarily five introductions (milo, kafir, hegari, feterita, and durra) or segregates from natural and deliberate crosses made from these introductions. Though the contribution of exotic germplasm has increased since the pre-hybrid era—76% of recently released parental lines contain contributions from exotic germplasm—the predominance of SC170 (PI534157) and SC110 (PI533794) in presently released parental lines indicated the continued need to diversify the sorghum germplasm pool (Duncan et al. 1991).

The U.S. National Plant Germplasm System (NPGS) maintains over 40 000 sorghum accessions. Despite its importance to the U.S. agricultural system, comprehensive curation of the crop did not begin until 1992. New agronomic descriptors have been adopted to further classify the collection (Dahlberg and Spinks 1995). The collection is too large to adequately screen at one time. Furthermore, the taxonomic classification and agronomic characterization of the total collection will require several more years.

A core collection of primarily exotic sorghum germplasm has the potential to improve on the efficiency of its use in accession characterization, evaluation, and utilization. A core collection could possibly benefit breeders within the United States by providing a sub-set of sorghums from different areas of the world that have been carefully described and characterized. This sub-set would provide commercial and public breeding programs with useful character-

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istics concentrated into groups based on country of origin, elevation, and other pertinent traits. A dynamic core collection will be a representative of the entire collection and will serve to improve the cost efficiency and maintenance of the sorghum collection within the NPGS.

Sorghum is a diverse crop taxonomically. Cultivated sorghums are classified within the subgenera *Sorghum.* Snowden (1936) presented the most complete classification of these sorghums. Because this classification was so complex, Murty et al. (1967) proposed using "head opening" to divide sorghum into eight sub-series with 70 preliminary working groups, which Murty and Govil later described (1967). Harlan and de Wet (1972) proposed a more simplified classification scheme of cultivated sorghums. Most breeders have come to recognize the usefulness of Harlan and de Wet (1972); however, because it does not fully describe the complexity of the species, they also use the working groups as described by Murty and Govil (1967). Dahlberg (2000) proposed an integrated classification system for sorghum that merged the race and working group classification into an updated classification structure. Race and working group classification of the U.S. collection began in 1992.

The diversity of the World Sorghum Collection has been documented (Mengesha and Rao 1982; Miller 1968). Webster (1976) outlined how that collection was assembled at ICRISAT in cooperation with the Rockefeller Foundation. By the end of the pre-hybrid era within the United States (1957), approximately 13 764 accessions had been introduced into the United States. Since that time, the NPGS has added another 18 841 accessions (as of September 1989) for a total of 32 605 accessions introduced into the United States (Duncan et al. 1991). Since that time, 8368 additional new sources of germplasm have been obtained for a total of 40 973 accessions.

Brown et al. (1987) developed the first core collection from the Australian collection of perennial *Glysine* spp. Later, Brown (1988) argued "that a better collection is one that is rationalized, refined and structured, around a small, well-defined and representative core." Frankel (1984) suggested that a core collection represents, "with a minimum of repetitiveness, the genetic diversity of a crop species and its relatives." Using various statistical and clustering approaches, core collections have been developed for several species such as annual Medics (Bauchan et al. 1992), perennial *Medicago* (Basigalup 1991; Basigalup et al. 1995), annual *Medicago* (Diwan et al. 1994), *Arachis hypogaea* (Holbrook et al. 1993), and the ICRISAT sorghum collection (Prasada Rao and Ramanatha Rao 1995).

Ideally, a sorghum core collection could be achieved by measuring the genetic variability within each accession through biotechnological techniques such as SSR, ISSR, RFLP, and RAPD analyses and maximizing the genetic variability of the core based on their results. These techniques have been used to determine genetic relationships in many crops (Chalmers et al. 1992; Schnell et al. 1995; Skroch and Nienhuis 1995; Welsh et al. 1991; Yang et al. 1996). Each paper provided information that supported the use of molecular markers in the identification of unique cultivars or populations based on genetic diversity. For large collections, however, the use of such techniques is cost prohibitive and difficult, and alternative strategies are required. The objectives of this research were to establish an initial core collection in sorghum and use clustering techniques to refine one sub-set of that core from the Sudan collection. The sub-set was then evaluated by measuring the genetic variability within each accession for metabolic sensitivity to heat stress.

Materials and Methods

Initial Development of Total Core Collection

The collection was first broken out into respective countries of origin. Using this criteria, 8000 accessions were identified as "Unknown" in their origin, and steps are underway to verify and update these "Unknown" fields. These accessions were not included in the initial core but may be added later as the geographical origins of these accessions are resolved. Accessions identified as being "breeder's material" or "improved" were also excluded from the core. The remaining accessions were stratified based on geography, and the core was developed based on a random selection of 10% of the accessions from each country.

Refined Core Collection from the Sudan

The sorghum collection from the Sudan has been well characterized. In 1993, 3182 acces-

sions from the Sudan were grown in a one-time quarantine regeneration at the Germplasm Introduction Research Unit, St. Croix, USVI. Data on 39 agronomic descriptors (see Dahlberg and Spinks 1995) were taken on 2924 plots that produced plants and data were uploaded onto the Germplasm Resource Information Network (GRIN). This data can be accessed at http://www.ars-grin.gov/npgs/searchgrin.html. Using imaging techniques described by Dahlberg and Wasylikowa (1996), sorghum kernels for each accession were analyzed for morphological differences. Six measurements, ArArea, ArBreadth, ArCircularity, ArMajor Axis Length, ArPerimeter Length, and ArRectangularity (BioScan, Inc. 1992), were taken and stored on a data sheet. Means from five samples were taken for each accession.

Nine quantitative data points were taken from the agronomic descriptors recorded in St. Croix. These included short-day anthesis, long-day anthesis, plant height, panicle exsertion, panicle height, rust (*Puccinia purpurea* Cooke) and anthracnose (*Colletotrichum graminicola* [Ces.] G. W. Wilson) ratings, yield potential, and desirability ratings. Desirability ratings are a ranking of overall plant characteristics and yield potential, with 1.0 being the most desirable plant and yield type and 5.0 representing the least desirable. These nine characteristics were merged with the seed morphological data to produce a database containing 14 quantitative measurements for each accession.

Of the 2924 accessions from which data were collected, 371 accessions were discarded in the consideration of core refinement because they were classified as improved, breeding lines. This left a total of 2553 accessions from which a refined core from Sudan could be developed. These accessions were first stratified based on their working group classification. Working groups, as defined by Dahlberg (2000), describe taxonomic variation within sorghum and are a logical base to use in clustering sorghums. The accessions within the Sudan collection were represented by 50 of the 68 working groups (Table 1). Twenty-seven of the working groups had ten or fewer accessions and these accessions were all selected as part of the refined core. Working groups with more than ten accessions assigned to them were clustered and analyzed (Dahlberg et al. 2002). The data set developed for each accession was standardized for each variable using z-scores. This data was then clustered, using PROC CLUSTER in SAS (1988) and random selections were taken from each cluster. This information was merged with the core developed from the 27 previously described working groups to form the refined core from Sudan.

Physiological Analysis of Vegetative High Temperature Sensitivity within the Sudan Sub-Set

Three measures of vegetative high temperature sensitivity were evaluated in this study. These include two assays that measured the inherent high temperature sensitivity of seed and leaf tissues without prior exposure to elevated temperatures (Imbibitional High Temperature Sensitivity Assay and Leaf High Temperature Sensitivity Assay), and one assay that evaluated a high temperature protection system induced by elevated, sublethal temperatures (Acquired Thermotolerance Assay). These assays were developed to provide an overall measure of how well a sorghum line copes with high temperatures from the time that water is first imbibed into the seed to the metabolic status of the leaves before and after exposure to high temperatures. Because the timing and magnitude of high temperature events are unpredictable across environments, it is hoped that these three assays will provide a clearer indication of the relative high temperature sensitivities of germplasm to be used in sorghum improvement programs.

Imbibitional High Temperature Sensitivity: Arabaskets[2] (Lehle Seeds, Round Rock, TX) were filled with dry soil (Sunshine Mix #3, Sun Gro Horticulture Inc., Bellevue, WA) packed well enough so that the soil did not sift out of the pots. Five seeds from each line representing the Sudan Core were placed on top of the soil in an individual Arabasket. The Arabaskets were transferred to an aquarium (~50 × 25 cm) located in a 40°C E15 Plant Growth Chamber (Controlled Environments Limited, Winnipeg, Manitoba, Canada), being careful that the seeds and soil were not disturbed. Forty degree Celsius water containing Peters Professional water soluble fertilizer (0.95 g/l 5-11-26 HYDRO-SOL [Scotts-Sierra Horticultural Products Company,

[2] Mention of a commercial or proprietary product does not constitute an endorsement by the USDA. USDA offers its programs to all eligible persons regardless of race, color, age, sex, or national origin.

TABLE 1. BREAKDOWN OF SUDAN SORGHUM COLLECTION SHOWING RACE DESIGNATION (HARLAN AND DE WET 1972), WORKING GROUP DESIGNATION (MURTY AND GOVIL 1967), TOTAL NUMBERS OF ACCESSIONS REPRESENTED WITHIN EACH WORKING GROUP, NUMBER OF ACCESSIONS DESIGNATED AS PART OF THE REFINED CORE, AND PLANT INTRODUCTION (PI) NUMBER FOR EACH ACCESSION DESIGNATED IN THE REFINED CORE.

Race	Working group	Total	Core	PI designation
Bicolor	Dochna-leoti	1	1	PI571371
Bicolor	Nervosum-broomcorn	1	1	PI568476
Bicolor	Sudanense	1	1	PI570498
Caudatum	Caudatum-kaura	1	1	PI570265
Caudatum	Dochna-nigricans	1	1	PI570926
Caudatum-Bicolor	Dochna-collier	1	1	PI568483
Durra-Bicolor	Subglabrescens	1	1	PI562924
Guinea	Margaritiferum	1	1	PI569053
Kafir	Caffrorum-durra	1	1	PI568650
Shattercane	S. verticilliforum	1	1	PI571360
Caudatum-Bicolor	Nigricans-bicolor	2	2	PI568326, PI568674
Durra	Dochna-durra	2	2	PI570431, PI568299
Guinea-Caudatum	Sumac	2	2	PI562941, PI570513
Guinea-Durra	Durra-roxburghii	2	2	PI568324, PI568325
Guinea-Kafir	Caffrorum-roxburghii	2	2	PI569066, PI568585
Guinea-Kafir	Roxburghii-shallu	2	2	PI570300, PI568310
Kafir	Caffrorum	2	2	PI569043, PI568654
Durra-Bicolor	Durra-dochna	3	3	PI569947, PI570410, PI570415
Kafir	Caffrorum-feterita	3	3	PI570921, PI568632, PI570933
Kafir-Caudatum	Caffrorum-birdproof	3	3	PI570318, PI568457, PI568536
Durra	Cernuum	4	4	PI571023, PI217708, PI217765, PI569411
Durra-Caudatum	Durra-feterita/Kaura	4	4	PI569037, PI569038, PI569039, PI569060
Guinea	Guineense	4	4	PI570748, PI291027, PI569014, PI570590
Kafir-Caudatum	Caffrorum-darso	4	4	PI562942, PI570823, PI570937, PI570330
Durra	Nandyal	6	6	PI217838, PI217891, PI569069, PI569219, PI568509, PI568657
Guinea-Durra	Durra-membranaceum	7	7	PI568523, PI568527, PI568528, PI568553, PI568581, PI568582, PI152736
Bicolor	Dochna	9	9	PI570695, PI571108, PI571109, PI569010, PI571374, PI569012, PI570372, PI568338, PI568446
Durra	Membraneceum	11	3	PI152728, PI568492, PI570280
Shattercane	Grass grain	11	3	PI569803, PI570917, PI570919
Bicolor	Bicolor-kafir	13	3	PI568373, PI568447, PI571234
Kafir-Bicolor	Caffrorum-bicolor	14	3	PI569248, PI570821, PI571020
Caudatum	Nigricans-feterita	16	3	PI569451, PI570509, PI571291
Durra-Caudatum	Nigricans-durra	16	3	PI568535, PI568545, PI569244
Durra-Bicolor	Durra-bicolor	17	2	PI568308, PI570443
Guinea	Conspicuum	21	4	PI569463, PI570685, PI571027, PI571349
Bicolor	Bicolor	22	3	PI563146, PI570253, PI570281
Kafir-Durra	Durra-kafir	25	4	PI568514, PI569150, PI569211, PI569306
Caudatum	Nigricans	26	4	PI563311, PI568670, PI570382, PI570708
Mixed	Mixed	35	5	PI569130, PI569848, PI568422, PI570477, PI570856
Caudatum	Dobbs	37	4	PI570424, PI568374, PI568605, PI570968

TABLE 1. CONTINUED.

Race	Working group	Total	Core	PI designation
Caudatum	Durra-nigricans	55	6	PI570394, PI568331, PI568517, PI568592, PI568621, PI570427
Guinea-Caudatum	Nigricans-guineense	58	5	PI568335, PI570096, PI570305, PI570321, PI570711
Durra	Durra	91	14	PI152654, PI152739, PI217768, PI54484, PI563379, PI568267, PI568292, PI569207, PI569995, PI570586, PI570720, PI571011, PI571045, PI571278
Caudatum	Zerazera	114	14	PI217799, PI568990, PI569120, PI569380, PI569430, PI569439, PI570335, PI570356, PI570702, PI570776, PI570940, PI570997, PI571090, PI571359
Caudatum-Bicolor	Caudatum-bicolor	123	12	PI568436, PI568468, PI568571, PI569032, PI569251, PI569332, PI569957, PI570259, PI570405, PI571190, PI571367, PI571380
Caudatum	Caudatum-nigricans	174	18	PI217837, PI568669, PI569132, PI569879, PI569917, PI569993, PI570059, PI570200, PI570378, PI570463, PI570705, PI570824, PI570942, PI571069, PI571124, PI571259, PI571293, PI571333
Caudatum	Caudatum-guineense	219	22	PI152615, PI563145, PI568312, PI568398, PI568402, PI569342, PI569343, PI569370, PI569416, PI569438, PI569450, PI570072, PI570104, PI570293, PI570348, PI570373, PI570504, PI570712, PI570802, PI570878, PI570929, PI571152
Caudatum	Caudatum-durra	250	29	PI217855, PI568371, PI568557, PI568579, PI569026, PI569062, PI569085, PI569110, PI569159, PI569273, PI569346, PI569861, PI570140, PI570160, PI570162, PI570213, PI570218, PI570267, PI570393, PI570434, PI570485, PI570549, PI570855, PI570923, PI570928, PI570943, PI571017, PI571155, PI571386
Caudatum	Caudatum-kafir	397	42	PI152612, PI562943, PI563262, PI568288, PI568319, PI568380, PI568382, PI568418, PI568432, PI568480, PI568500, PI568567, PI568664, PI569022, PI569082, PI569094, PI569100, PI569106, PI569113, PI569116, PI569129, PI569151, PI569160, PI569163, PI569220, PI569234, PI569253, PI569274, PI569277, PI569291, PI569357, PI569377, PI570061, PI570157, PI570221, PI570258, PI570285, PI570383, PI570500, PI570602, PI571351, PI61455

TABLE 1. CONTINUED.

Race	Working group	Total	Core	PI designation
Caudatum	Caudatum	737	75	PI152611, PI152703, PI217781, PI563299, PI563308, PI563319, PI563323, PI568328, PI568351, PI568352, PI568364, PI568414, PI568534, PI568667, PI569272, PI569295, PI569816, PI569823, PI569856, PI569860, PI569876, PI569880, PI569894, PI569897, PI569904, PI569921, PI569922, PI569938, PI569978, PI570000, PI570123, PI570174, PI570181, PI570211, PI570453, PI570469, PI570532, PI570548, PI570552, PI570688, PI570735, PI570740, PI570769, PI570773, PI570782, PI570784, PI570787, PI570815, PI570818, PI570825, PI570830, PI570833, PI570837, PI570838, PI570876, PI570888, PI570896, PI570939, PI570944, PI570974, PI571007, PI571040, PI571112, PI571116, PI571164, PI571169, PI571212, PI571215, PI571218, PI571267, PI571269, PI571275, PI571325, PI571341, PI571355
	Totals	2553	352	

Maryville, OH], supplemented with 0.475 g/l calcium nitrate [Ca Hydro Agri North America, Inc., Tampa, FL], and 0.238 g/l magnesium sulfate [Scotts-Sierra Horticultural Products Company, Maryville, OH]) was added slowly to the aquarium by pouring it against the side of the container to a depth of 2 cm. If care was not used, the baskets would tip over and/or the soil would sift out of them. The soil was allowed to soak up the water for approximately 30 minutes before adding more solution to the aquarium. This was repeated until the soil was completely wet. Then, enough solution was added so that the baskets sat in 1.5 to 2 cm of nutrient solution (~1 L). Once the soil was completely wet, additional soil was sprinkled over the seeds in order to cover them. Each aquarium was filled with solution daily to a 1.5 to 2 cm depth. The plants remained in the growth chamber under constant incandescent and fluorescent lighting (190 μmol m^{-2} s^{-1}) for five days prior to analysis. Color photographs were taken of each line in the Arabaskets and following removal of the soil to reveal the root system. Measurements of shoot and root lengths of each plant were obtained.

Leaf High Temperature Sensitivity: Seeds of lines within the Sudan core collection were planted in1 gallon pots containing Sunshine Mix #3 (Sun Gro Horticulture Inc., Bellevue, WA) and seedlings were grown under greenhouse conditions (28 ± 5°C air temperature) for 30 to 60 days. Leaf samples were harvested in mid- to late-afternoon using a #6 cork borer. The harvested leaf punches were placed in a beaker of water until they could be transported to the laboratory. Temperature treatments were achieved using an electronically controlled eight position thermal plate system. Thermal plates were covered with water-saturated 3MM filter paper on which the leaf punches were placed. The leaf punches were covered with Glad Wrap® (a gas permeable plastic membrane) to prevent the 3MM paper from drying. Initial studies evaluated the effect of exposing the leaf punches to a range of incubation temperatures in the dark on subsequent Photosystem II variable fluorescence. Photosystem II variable fluorescence was

obtained from the leaf punches using either a Plant Efficiency Analyser and/or Handy-PEA (Hansatech Instruments, PP Systems Inc., Haverhill, MA). Initial incubation temperatures of 32, 34, 36, 38, 40, 42, 44, and 46°C were evaluated. Subsequent experiments evaluated plant responses to a 32 and 42°C incubation prior to fluorescence analysis.

Acquired Thermotolerance: The assay for acquired thermotolerance is based upon the inhibition of chlorophyll accumulation in continuous light following heat treatment of the etiolated material. The temperature that provided maximum chlorophyll was identified before evaluating the acquired thermotolerance characteristics of the sorghum lines. Leaf segments (1 cm section taken 1 cm from the leaf tip) from seedlings grown for five to six days under a continuous cycling of two minutes light from a 25 W incandescent bulb followed by 118 minutes of darkness were exposed to continuous illumination at 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, or 45°C for 20 h. Chlorophyll accumulation was determined following the light treatment by the procedure of Arnon (1945) or by use of a Minolta SPAD 502 chlorophyll meter. The challenge temperature, a 30 minute temperature exposure that prevented subsequent chlorophyll accumulation, was determined by placing leaf segments of the intermittent light-grown sorghum on moistened 3MM paper and incubating them in the dark for 30 minutes at 30°C (control), 44°C, 46°C, 48°C, 50°C, 52°C, 54°C, or 56°C. Following the 30 minute temperature challenge the leaf segments were given a 20 h light treatment at the optimal temperature for chlorophyll accumulation and the chlorophyll content of leaf segments determined as described above. The temperature exposure that inhibited chlorophyll accumulation by more than 90% of the control chlorophyll level was chosen as the challenge temperature for subsequent germplasm evaluation. Burke (1994, 1998) demonstrated that a 4 h pre-incubation at an elevated, sub-lethal temperature could reduce the level of injury experienced by plants subsequently exposed to a formerly lethal challenge temperature. To determine the appropriate pre-incubation temperature for sorghum, intermittent light-grown sorghum leaf segments were pre-incubated for 4 h at 30°C (control), 34°C, 36°C, 38°C, 40°C, 42°C, 44°C, or 46°C. Following the pre-incubation period, the leaf segments, with the exception of the 30°C control, were exposed to the predetermined 30 minute challenge temperature. The control and challenged leaf segments were placed at 30°C for 20 h under continuous light and subsequent chlorophyll accumulation evaluated as described above. Seedlings from the Sudan core collection were screened initially for acquired thermotolerance diversity by pre-incubating leaf segments at the identified pre-incubation temperature for 4 h then challenging the segments at the challenge temperature for 30 minutes. The leaf segments were then transferred to continuous light at 30°C after which chlorophyll content was determined as described above.

RESULTS AND DISCUSSION

An initial core collection for sorghum was developed through stratification based on country of origin and random selection of approximately 10% of the accessions within each country. The core is composed of 3011 accessions representing 77 different countries. Their designation has been placed on GRIN (see http://www.ars-grin.gov/cgi-bin/npgs/html/desclist.pl?69). Of the 3011 accessions designated as part of the initial core subset, only 2443 are currently available within the system. The accessions not available either have limited seed quantity, poor seed viability, or are still within the quarantine system. These accessions will be targeted for a special regeneration so that they can become available for distribution in the near future.

Refined Core Collection from the Sudan

The refined core collection from the Sudan contains 71 accessions from the 27 working groups with less than ten accessions per working group (Table 1). Using the working group zerazera as an example, the 23 other working groups were evaluated as follows. Data from the working groups were standardized and Ward's minimum variance cluster analysis was used to develop clusters within each dataset (SAS 1988). From this, it was determined that the zerazeras could be broken into 12 distinct clusters (Table 2). Approximately 10% of the accessions assigned to each cluster were then randomly selected to develop a refined core of zerazeras equaling 14 accessions (Table 1). Selections from the other 22 working groups were carried out in similar fashion to produce an additional

TABLE 2. ASSIGNMENT OF ACCESSIONS TO EACH OF 12 CLUSTERS BASED ON WARD'S MINIMUM VARIANCE CLUSTER ANALYSIS FROM THE WORKING GROUP ZERAZERA, SUDAN COLLECTION. FROM EACH CLUSTER, APPROXIMATELY 10% OF THE ACCESSIONS WERE RANDOMLY SELECTED TO PRODUCE A REFINED CORE SUBSET.

Cluster #	Accession identifiers
*	PI571096, PI571097, PI571151, PI569380, PI568594, NSL52094, PI217879, PI267474,
1	PI570779, PI570995, PI286245, PI570982, PI217707, PI570940,
2	PI570997, PI570998, PI570312, PI568538, PI571191,
3	PI569905, PI569908, PI569896, PI569899, PI570862, PI570743, PI569900, PI570308, PI570776, PI57099b, PI571201, PI569903, PI569919, PI569907, PI568990, PI571000, PI570052, PI570056, PI569969, PI570241, PI571303,
4	PI570340, PI570578, PI569976, PI570335, PI569430, PI569431, PI569977, PI570193, PI570984, PI570313, PI570299, PI569961, PI570329, PI571283, PI570355, PI570320, PI570593, PI570216, PI570057, PI570369,
5	PI570357, PI570575, PI571359, PI571276, PI570347, PI568556, PI570323, PI570354,
6	PI570327, PI570345, PI570717, PI570298, PI570844, PI570588, PI568539, PI568601, PI571090, PI570346, PI569998, PI570755, PI570739,
7	PI569968, PI570356, PI569140, PI569304, PI569226, PI569289, PI571038, PI569982, PI570344, PI569925, PI562927, PI569351,
8	PI570777, PI569087, PI570702, PI570337, PI570799,
9	PI570349, PI568372, PI569167, PI570018, PI568407, PI570780, PI568615, PI569383, PI217799, PI570726, PI570251,
10	PI568336, PI570577, PI57099a, PI569439,
11	PI569120,

* = accessions that did not have complete data sets. The accession PI57099 had two forms, a & b, that were different in field evaluations.

281 accessions. These were added to the 71 accessions to produce a total of 352 accessions that represent 13.8% of the total collection from Sudan (Table 1).

IMBIBITIONAL HIGH TEMPERATURE SENSITIVITY

In the first experiment the 352 accessions were planted in soil and moistened with room temperature water prior to being moved to the growth chamber. Planting took approximately 6 h and then the accessions were moved to the growth chamber. Seedling emergence from the soil occurred in 313 of the 352 accessions and the seedlings were sorted into distinct groupings based upon seedling height (tall versus short) and chlorophyll accumulation (green leaves, yellow leaves, or leaves with some green and some yellow regions). During the course of the analysis we became concerned that some of the diversity observed might have arisen from the different length of time that the accessions were in moist soil at room temperature. To remedy this potential problem, subsequent plantings were placed in dry soil and all pots were moistened simultaneously with 40°C water in the 40°C growth chamber. This procedural change resulted in a reduction in the number of accessions that emerged from the soil from 313 in experiment 1 to 171 accessions in subsequent experiments.

These results suggest that the ability to mobilize stored reserves impacts the phenotypic response of the seedling. In a similar study of imbibitional heat tolerance of sorghum hybrids we found that germination for 24 h at 29°C could completely protect against the heat-induced damage observed in seedlings that imbibed 40°C water (data not shown). The 171 accessions that germinated and emerged from the soil in the present study were used in subsequent experiments. The accessions were sorted into distinct groups as described in experiment 1 and their performances following two additional 40°C tests were evaluated. Figure 1 shows photographs that compare seedling growth at 29°C and 40°C for two of the accessions. Although similar growth characteristics were observed between PI570096 and PI570695 at 29°C, distinct differences in growth habit were observed when germinated at 40°C. PI570096 seedling photobleached upon emergence from the soil, while PI570695

Fig. 1. Photographs of two sorghum accessions (PI570096 and PI570695) grown under continuous 29°C (left) or 40°C (right) temperatures. Although similar growth habits were observed at 29°C, PI570096 exhibited severe heat injury upon emergence at 40°C, while PI570695 showed a more moderate reduction in shoot length and maintained high chlorophyll levels.

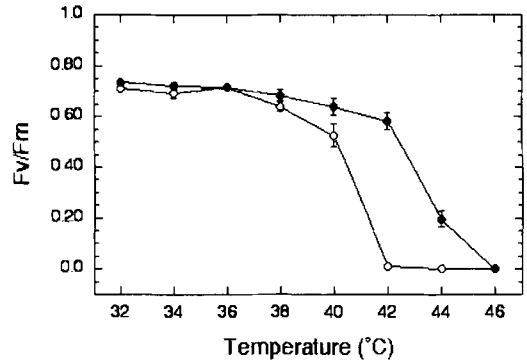

Fig. 2. A representative graph showing the effect of prolonged exposures to increasing temperatures on the ratio Photosystem II variable fluorescence (Fv) to the maximum fluorescence level (Fm) on two sorghum lines, the resistant line (closed circle) PI570477 and the susceptible line (open circle) PI570974. Maximum differences between the two lines were observed at 42°C.

emerged and maintained chlorophyll levels. Accessions that emerged from the soil and maintained green foliage in all experiments included PI558990, PI570181, PI570477, PI570504, PI570509, PI570688, PI570695, PI570702, PI570815, PI571152, and PI571386. These accessions represent three races and seven working groups within the core collection.

Leaf High Temperature Sensitivity

A second assay was used to evaluate the relative heat susceptibility of leaf tissues excised from greenhouse-grown PIs from the Sudan core collection. Initial studies evaluated the effects of exposing leaf punches to 32, 34, 36, 38, 40, 42, 44 or 46°C in the dark on subsequent measurements of Photosystem II variable fluorescence. Fluorescence response curves are shown for two sorghum lines (PI570477 and PI570974) in Fig. 2. The ratio of Photosystem II variable fluorescence (Fv) to the maximum fluorescence level (Fm) was determined for each incubation temperature. Fv/Fm ratios remain relatively constant with incubations from 32 to 38°C. Above 38°C the Fv/Fm ratio declined rapidly in one line (open circles) while the second line maintained elevated Fv/Fm ratios from 32 to 42°C. Temperatures above 42°C resulted in a rapid decline in Fv/Fm ratios in the second line. In subsequent experiments Fv/Fm ratios were determined only at 32°C and 42°C. The 32°C incubation was chosen as it showed no significant change in Photosystem II variable fluorescence following a 24 h incubation. The 42°C incubation was used as it provided the stress temperature that exhibited the greatest variability between samples. Analysis of the core collection revealed a broad range of thermal sensitivities for this parameter. Figure

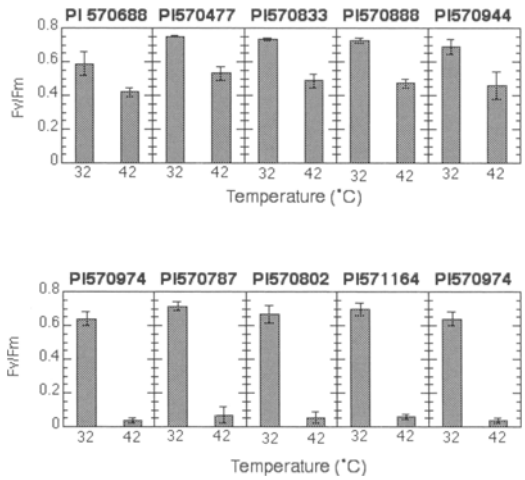

Fig. 3. Graphs showing five resistant and five sensitive accessions of the Sudan core collection based upon the effect of prolonged exposures to 42°C on the ratio Photosystem II variable fluorescence (Fv) to the maximum fluorescence level (Fm).

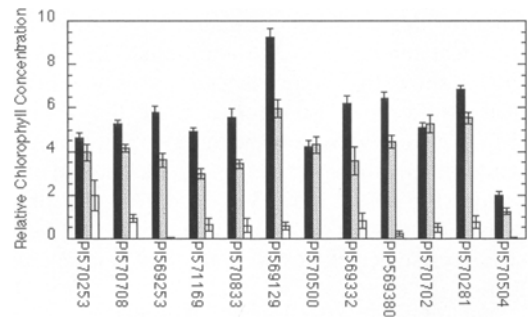

Fig. 4. Graph of chlorophyll accumulation at 30°C following a continuous 30°C incubation (black bar), a 4 h 40°C pre-incubation followed by a 30 min 48°C challenge (shaded bar), or a 30 min 48°C challenge (open bar). The level of acquired thermotolerance, measured as the level of chlorophyll accumulation following the 4 h 40°C pre-incubation followed by a 30 min 48°C challenge compared to continuous 30°C controls, ranged from 50 to 100% protection.

3 provides a graphical representation of five of the more heat resistant PIs and five of the most sensitive PIs within this collection for this particular metabolic assay. It is interesting to note that two of the imbibitional heat tolerant lines, PI570477 and PI570688, were also among the best lines in the leaf high temperature sensitivity assay. PI570833 and PI570888 showed excellent leaf heat resistance and also were ranked in the upper one third of the collection in the imbibitional heat tolerance assay. PI570944, however, showed excellent leaf heat resistance yet ranked in the lowest one fourth of the collection in the imbibitional heat tolerance assay. Those lines showing the most heat sensitivity in the leaf fluorescence assay also spanned from sensitive (PI570974) to resistant (PI570802) accessions in the imbibitional heat tolerance assay. These results support the concept that the imbibitional heat tolerance assay and the leaf high temperature sensitivity assay are independent assays, and that there is considerable genetic variability within the core collection for these metabolic parameters.

Acquired Thermotolerance

The diversity in acquired thermotolerance among the accessions within the core collection was evaluated using chlorophyll accumulation following heat manipulation. This procedure has been used to evaluate acquired thermotolerance across numerous species (Burke 1994, 1998; Burke et al. 2000; O'Mahony et al. 2000; O'Mahony and Burke 2000). Figure 4 illustrates a range of protection from approximately 50% to 100% protection against the high temperature challenge. High levels of protection were observed in PI570500 and PI570702, while low levels of protection were observed in PI569332 and PI571169.

A comparison of the relative responses of the sorghum accessions to the three high temperature assays is shown in Fig. 5. The results show that these assay systems evaluate distinct aspects of the sorghums' metabolism and that each as-

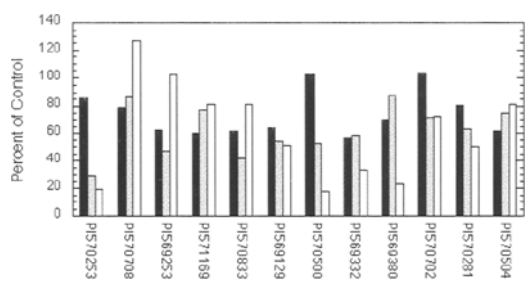

Fig. 5. Graph showing the relative level of heat tolerance determined from the three bioassays for a selected subgroup of the Sudan core collection accessions (black bar—acquired thermotolerance, gray bar—leaf heat resistance, open bar—imbibitional heat resistance [shoot length]). Significant genetic variability for heat tolerance can be seen across the accessions for these bioassays.

say provides unique information about the relative heat tolerance of the sorghum. The PIs in Fig. 5 show that some accessions only exhibit heat stress protection for one of the assays (PI570253) or two of the assays (PI569380), while others show protection in all three of the assays (PI570702, PI570504, PI570708). Clearly significant levels of genetic diversity for heat stress resistance exist within the Sudan core collection.

The ultimate impact of the sorghum core collection at this time is unknown. Sorghum is grown primarily in the semi-arid areas of the tropics and many of the sorghums that are grown are photoperiod sensitive—selection pressure from farmers has produced sorghums that flower traditionally at the end of the rainy season allowing for the grain to ripen under dry conditions (Doggett 1988). Because of this, individual cultivars or germplasm accessions respond differently to photoperiod and temperature regimes. The sorghum species has been classified as a short-day type. Miller et al. (1968) established that sorghum has a critical photoperiod of 12 hours. Several minutes' differences can affect duration of vegetative growth. Because sorghum is a short-day species, the introduction of exotic germplasm into the United States for use into breeding programs had been limited. This may also limit the usability of the sorghum core collection, however, steps have been undertaken to develop the core. Theoretically, the core should provide a road map from which scientists can map genetic diversity of sorghum and further enhance the capabilities to isolate and clone genes of importance in the future.

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